Chapter 6

Deficiency of Folate in Pregnancy on Diverse Subjects Using FTIR Spectroscopy

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Additional information is available at the end of the chapter

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Abstract

This study is an attempt to assess, evaluate and compare the spectral difference in saliva and serum between healthy and anomalies pregnant women because of deficiency of folate by utilizing Fourier Transform Infrared Spectroscopy. Folate is required for the development of healthy embryo and plays vital role in the fetus spinal cord and brain development. The present work is to study the folate deficiency in pregnancy-Anomalies (open neural defect) and contrast the outcome of the result with normal healthy pregnant women. The outcome of the results showed that there is a significant difference or contrast between the folate of healthy pregnant and anomalies (open neural defect) in pregnant women, both in the sample of saliva and serum. From the spectral analysis, the intensity ratio parameters have been computed and introduced. The result of the outcomes shows that for both qualitative and quantitative investigation of biological fluids and to distinguish between the sample sets from healthy and anomalies-diseased groups, FTIR is utilized. The internal standard method is described in characterizing the samples quantitatively.

Keywords: saliva, serum, folate deficiency, FTIR, pregnant women

1. Introduction

Historically, investigations of saliva of female sex hormones were utilized for fertility and pregnancy monitoring [1–3]. Changes in salivary pattern in each trimester of normal pregnant women have been compared by utilizing FTIR spectroscopy both qualitatively and quantitatively [4]. However, a recent finding shows that these assays might be helpful and useful

beyond the investigation of reproductive concerns. There is a clear picture that during pregnancy there are changes and hormone level fluctuations in normal pregnancy. In specific issues with the fetus during pregnancy complications, frequent serum sampling testing for hormone analysis is invasive, inconvenient, and requires skilled personal to draw samples. However, whole saliva provides an excellent sample to observe the hormone levels. Fourier Transform Infrared Spectroscopy (FTIR) is utilized to characterize the structural and chemical composition of human saliva of pregnant women. Each and every molecule excited to higher states of vibration using light at a particular wavelengths which corresponds to the excited vibration modes of frequency. This information or data can be utilized to outline or map the absorption positions and help to recognize in identifying the chemical properties of the tissue. FT-IR spectroscopy can provide unique infrared chemical fingerprints of specimens, highly sensitive which can yield new insights into positional salivary changes in pregnant women. A key advantage of FT-IR is that it is a non-manipulative, quick and non-invasive collection method, simple transport of the material, easy and no additional need for media which can identify a wide range of chemical targets (**Figure 1**).

Saliva, similar to blood, contains protein and nucleic acid molecules which are vast, large and complex that reflects the physiological status. Essential folate consumption, during the period of preconception, is the perfect time when a woman becomes pregnant and it helps to protect against numeral congenital deformities, including neural tube defects which are the most prominent birth defects that happen from deficiency of folate [5]. Neural tube defects which

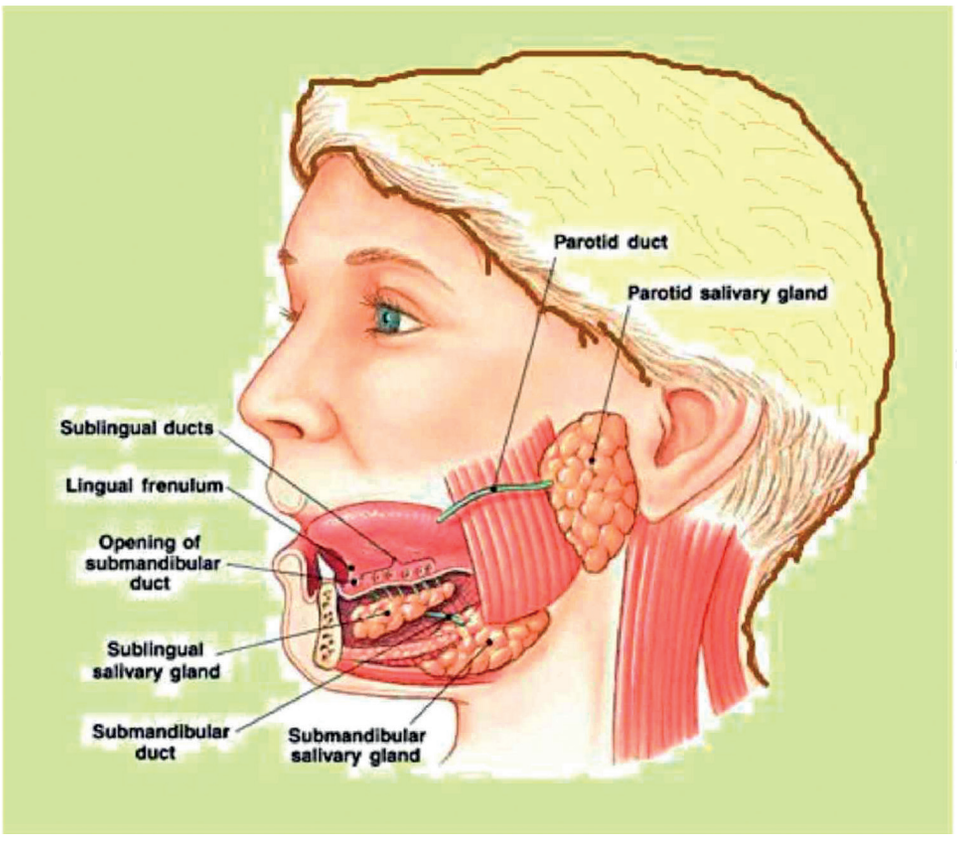

Figure 1. The salivary gland.

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cause critical abnormalities of the central nervous system that procure in babies during the initial stage or first few weeks of pregnancy, terminating in malformations of skull, spine and brain. The normal defects in neural tube are anencephaly and spina bifida. The risk of neural tube defects is reduced in a critical way when additive folate is devouring into a healthy diet before conception and during the 1st month after conception [6, 7]. Folate supplements has additionally been appeared to lessen the risk of congenital heart defects, limb defects, cleft lips [8] and urinary tract irregularities [9]. Folate deficiency during pregnancy may likewise raise the risk of preterm delivery, spontaneous premature birth, baby low birth weight and retardation of fetal growth and complications in pregnancy, as abruption placental and pre-eclampsia [10]. Supplementation with Folate may likewise ensure the fetus against disease when the mother is battling disease or taking prescriptions or smoking during pregnancy [11]. It includes oocyte development, implantation, placentation, including the general impacts of folate and pregnancy. Consequently, it is important to get adequate and sufficient amounts through the routine diet to avoid from subfertility [12]. There is an improvement through worldwide that pre-birth high folate in perspective of low vitamin B_{12} resulting in epigenetic changes in the unborn predisposing them to grown-up onset of fetal cause infection or disease in particular metabolic disorders, central adiposity and adult illnesses, as Type 2 diabetes [13]. Moreover, another dynamic area of research and concern is that either substantially more or too little folate in utero affects epigenetic changes to the brain bringing about a mental imbalance as autism spectrum disorders [14, 15]. A salivary test is more secure and safer than utilizing serum. The noninvasive collection approach incredibly increases their readiness or willingness to experience health inspections, wellbeing reviews, it reduces uneasiness, tension and monitors their general health wellbeing over time and helps in diagonose morbidities in the beginning period that is the early stage. Utilizing an effective assay and successful measure, salivary diagnostics assumes a vital role in routine monitoring the health and observing the early disease detection [16]. In mid IR spectroscopy, the pure folate is described with hydroxyl stretching and stretching of NH vibrations, the bond of C=O for stretching vibration of carboxyl group, and the bond of C=O stretching vibration of $–CONH_2$ group and bending of NH vibration [17]. A connection has additionally been confirmed between neural tube defects in human and defective metabolism of folate [18, 19]. Furthermore, an association has been described between neural tube defects.

2. Pregnancy

Pregnancy is the development and fertilization of one or more offspring, known as fetus or an embryo, in a woman's uterus. In a pregnancy, there can be various multiple gestations, as on account of triplets or twins. Childbirth generally occurs around 38 weeks after origination [20]; in women who have a menstrual cycle length of a month, this is roughly 40 weeks from the begin of the last normal ordinary menstrual period (LMP). Human pregnancy is the most studied of every single mammalian pregnancy.

The term embryo is utilized to describe the developing offspring during the initial 2 months following conception. The term fetus is utilized from 2 months until birth.

In many societies' medical or legal definitions, human pregnancy is somewhat arbitrarily divided into three trimester periods as a means to simplify the different stages of prenatal development. The first trimester carries the highest risk of miscarriage (natural death of embryo or fetus). During the second trimester, the development of the fetus can be more easily monitored and diagnosed. The beginning of the third trimester often approximates the point of viability, or the ability of the fetus to survive, with or without medical help, outside of the uterus.

3. Serum

In blood, serum does not contain white or red blood cells and it is the serum component which is neither a blood cell nor a clotting factor; it is the blood plasma with the removed fibrinogens. Serum which includes all proteins not utilized as a part of blood clotting that is coagulation and all electrolytes, antibodies, hormones, antigens, and any exogenous substances such as microorganisms and drugs. An investigation of serum is serology, and may likewise include proteomics. Serum is utilized as a part of numerous diagnostic tests, and also in addition blood typing (**Figure 2**).

The blood is centrifuged to remove cellular components. Anti-coagulated blood which yields plasma containing clotting factors and fibrinogen. Coagulated blood (thickened or clotted blood) yields serum without fibrinogen, and remain some clotting factors.

Serum is a basic and essential factor for the self-renewal of embryonic stem cells which is in combination with the cytokine leukemia inhibitory factor. The clear fluid that can be isolated from coagulated blood. Serum differs or contrasts from plasma, the portion of liquid of normal unclotted blood containing the white and red cells and platelets. The coagulation which makes the difference between plasma and serum. The "Serum" which includes maternal serum, serum glutamic oxaloacetic transaminase (SGOT), alpha-fetoprotein (MSAFP), serum glutamic pyruvic transaminase (SGPT), and serum hepatitis.

The term "serum" is also used to designate any normal or pathological fluid that resembles serum as, for example, the fluid in a blister.

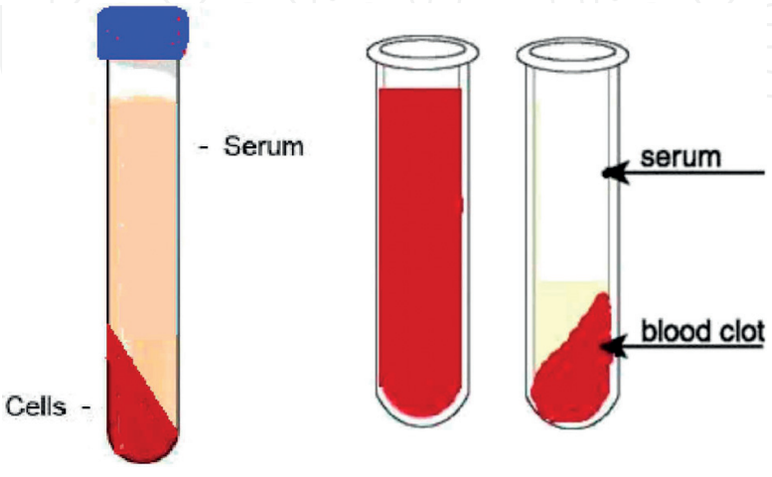

Figure 2. Serum.

4. Deficiency of folate

Folic acid is called as vitamin B-9 or folate. In the B-complex family, it is a water-solvent vitamin. Everybody needs a diet which includes folate, regardless of whether or not they are pregnant, as inadequacy of folate leads to medical or health issues [20]. However, adequate intake of folate is considered particularly during pregnancy.

4.1. Folate and neural tube defects

The most effective argument for pregnant women requiring folate supplements originates from the link between adequate intake of folate and reduced risk of having a child with defects in neural tube. Defects in Neural tube are a classification of congenital defects in birth which is affecting the spinal cord and the brain, the most widely common being is anencephaly and spina bifida. Defects in neural tube can be seriously disabling or fatal for a baby development.

In addition, there has been some clear evidence that folate may decrease the risk of other birth defects too, and that a mother with low folate may likewise have a higher risk of miscarriage, placental abruption, preclampsia and preterm delivery due to the connection between low folate and homocysteine levels [10, 20–24].

5. Fourier transform infrared spectroscopy (FTIR)

The preferred method of infrared spectroscopy is FT-IR, and it stands for Fourier Transform infrared, in Infrared Spectroscopy, in which the infrared radiation is passed through a sample. A portion of the infrared radiation is absorbed by the sample and some of it is transmitted. The spectrum resulting, represents the transmission and molecular absorption which makes a molecular finger print sample. Like a unique mark of fingerprint no two one of a kind molecular structures which produces the similar infrared range of spectrum. This makes infrared spectroscopy valuable for various investigation. Information provided by FTIR,

- It can identify unknown materials,
- It can determine the quality or consistency of a sample and
- It can determine the amount of components in a mixture

The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical algorithm) is required to convert the raw data into the actual spectrum.

5.1. Developmental background

In 1957, the first low cost spectrophotometer capable for recording an infrared range of spectrum was the Perkin Elmer Infrared. This instrument covered the range of wavelength from 2.5 to 15 μm (range of wave number 4000–660 cm^{-1}). Later instruments utilized potassium bromide crystals to stretch out or extend the range to 25 μm (400 cm^{-1}) and cesium

iodide 50 μm (200 cm^{-1}). Beyond the region 50 μm (200 cm^{-1}) prominently known as the region of far-infrared, at long wavelengths it converges into the region of microwave.

5.2. Fourier transform infrared (FTIR) spectrometers

Fourier change spectrometers have replaced recently dispersive instruments for most applications because of their predominant speed and sensitivity or affectability. They have extraordinarily expanded the capacities of infrared spectroscopy and have been applied to various or many areas that are exceptionally difficult or about difficult to analyze by dispersive instruments. Rather than viewing each and every component frequency sequentially, as in a dispersive Infrared spectrometer, all frequencies are inspected and examined simultaneously in Fourier Transform Infrared (FTIR) Spectroscopy. FTIR depends on the basic or fundamental principles of molecular spectroscopy. The multitude of experimental techniques some of which are found in other oil tests analysis, and others that are sophisticated to the point that they are of significance just in look into research facilities.

The essential rule or the basis principle behind molecular spectroscopy is that the molecules which are specific, absorbs light energy at particular wavelengths, known as their resonance frequencies. For instance, the molecules of water resonates around the 3450 wavenumber (given the symbol cm^{-1}), in the region infrared of the electromagnetic range of spectrum.

A FTIR spectrometer works by taking a sample of small quantity and introduce it with the infrared cell, and it is subjected to light source of infrared scanned from 4000 cm^{-1} to around 600 cm^{-1}. The light intensity transmitted through the sample is measured at each wavenumber which allows the amount of light absorbed by the sample to be resolved as the contrast between the light intensity after and before the sample cell. This is known is the infrared range of spectrum of the sample.

A wavenumber, given the symbol of cm^{-1}, is just the reverse or inverse of the wavelength of the light. For instance, 3450 cm^{-1}, the resonance frequency of water which corresponds to the wavelength of light of 0.00000290 or 2.9×10^{-6} m, in the region of infrared of the electromagnetic spectrum. As opposed to utilizing the cumbersome unit of 10^{-6} m, spectroscopists basically take the inverse to give a number which is easier and more helpful to utilize.

In the infrared region of the range of spectrum, the molecule of resonance frequencies of an atom due to the presence of functional group molecule which is specific to the molecule. A functional group is a group of two or more atoms bonded together in a particular way.

6. Research design and data collection

6.1. Spectrum One FT-IR spectrometer (Perkin-Elmer)

In infrared Spectroscopy, the PerkinElmer Spectrum 100 Series FT-IR spectrometers are the highest quality level in testing materials, academia and in applications of research. The new Spectrum 100S version exhibits or demonstrates the highest sensitivity in its class which is

enabling much faster measurement of even the most samples demanding such as absorbing highly or poorly reflecting materials.

For the more cost-conscious laboratory, the Spectrum 100R combines ease of use, reliability, performance at a cost typically observed among instruments with a small amount of the 100R's systematic power.

Characterizing the standard for the technology of FTIR, for more than 60 years, PerkinElmer is an accomplished and knowledgeable supplier and experience of FTIR spectrometers for research facilities and laboratories around the world. By adopting a complete quality strategy - from design of product, development and assembling through client or customer service and support - PerkinElmer gives the most highest quality FTIR system of frameworks, alongside the most exact, accurate and reproducible outcomes in the industry.

The Perkin Elmer Spectrum 100 Series spectrometers are bench top instruments that provides all the following in one self-contained unit as shown in **Figure 3**. The sample compartment of a large, purgeable, the instruments which can operate in ratio, interferogram mode or in a single beam. An optical system that gives data collection over a total range of 7800–370 cm^{-1} (220 cm^{-1} with CsI beam splitter) with a best resolution of 0.5 cm^{-1} for the spectrum 10 FT-IR, a mid-infrared detector-either DTGS or $LiTaO_3$ (lithium tantalate) as standard and the using MCT (Mercury Cadmium Telluride) or PAS (a photoacoustic detector) option for the spectrum 100 FT-IR.

6.1.1. Software

Generally a single software platform incorporates all the functions required for infrared analyses; instrument control, data manipulation and analysis, and flexible report utilities. A suite of optional software packages provide advanced capabilities or functions designed for specific application areas.

Figure 3. The spectrum 100 series spectrometer.

6.1.2. Applications

FTIR can be used in all applications where a dispersive spectrometer was used in the past. In addition, the multiplex and throughput advantages have opened up new areas of application. These include: Micro-examples. The forensic analysis of tiny samples which can be investigated in the sample chamber with the aid of an infrared microscope Minor examples, the surface image can be analyzed with the scanning magnifying lens. A surface picture can be obtained by scanning [25]. Another case is the utilization of FTIR which is used to describe creative or artistic materials in old-master paintings [26].

In Emission spectra, rather than recording the range of light spectrum transmitted through the sample, FTIR spectrometer can be utilized to obtain range of light produced by the sample. Such emission or outflow could be incited by different processes, and the most widely recognized ones are Raman scattering and luminescence. Little change is required to an absorption of spectrometer of FTIR to record spectra of emission and along these numerous commercial FTIR spectrometers combine both emission/Raman modes and absorption [27].

In Photocurrent spectra, the mode utilizes a standard FTIR spectrometer absorption. The examined or studied sample is set rather than the FTIR detector, and its photocurrent, prompted by the spectrometer's broadband source, which is utilized to record the interferrogram, which is then changed over into the photoconductivity sample spectrum [28].

6.2. Data collection

The FTIR spectroscopic features of saliva and serum of normal and anomalies pregnant women – deficiency of folate, changes in the salivary hormones of normal and anomalies are discussed. Informed consent were obtained from all subjects as approved by local ethics committee. The saliva samples were collected from normal and anomalies pregnant women from Saveetha Hospital, at Chennai. Saliva samples were collected from 20 volunteers in each set. 5 mL saliva samples were obtained in a tube from each individual and then used for the spectral analysis. All the procedures and methods of sampling were performed between 12 p. m. and 1 p.m. The measurement of FTIR spectra were totally completed at Sophisticated Analytical Instrumentation Facility, IIT, Madras, Chennai-36, using range one PerkinElmer FTIR spectrophotometer. The spectra were recorded in the region of mid infrared of 4000–400 cm^{-1} in the absorption mode. 50 μL of each solution was spread evenly and uniformly on the window of crystal of thallium bromide. The samples were air dried for water evaporation to isolate out the stray out the bands of absorption due to water. The spectrometer is furnished or equipped with a globar source and DTGS cooled locator. The sampling window is scanned as the background and 32 scans are co-included with a spectral determination resolution of 1 cm^{-1}. All the spectra were corrected with baseline and it has been standardized to achieve the identical area under the curve.

Intensity ratio parameters are computed and it shows that the FTIR spectroscopy has been successfully applied in the study of analysis of saliva and serum of normal and anomalies pregnant women.

7. Vibrational analysis FTIR

The FT-IR spectra of saliva and the serum samples of anomalies and normal pregnant women demonstrates the corresponding absorption bands in their particular regions qualitatively. In any case of quantitatively, there is a considerable spectral distinction between the saliva of normal and anomalies pregnant women and the serum of normal and anomalies pregnant women. The absorbance is specifically or directly proportional to the concentration. Thus the serum and saliva sample of normal and anomalies pregnant women are investigated and analyzed quantitatively by calculating the intensity ratio among the peaks of absorption. The other region of 3600–3000 cm^{-1} includes C-H, O-H and N-H vibrations of stretching of proteins. Intermolecular hydrogen bond increases and the concentration increases as decreases in frequency and also the additional bands begin to appear and the region of 3550–3200 cm^{-1} is at the expense of the "free" hydroxyl band [29]. It is seen in the most part of the spectra that the vibrations across from stretching of N-H vibration and stretching of O-H vibrations got merged and demonstrated a single and broad curve in this region.

The band around the region 3500–2800 cm^{-1} is due to cholesterol, Phospholipids and creatine and vibrations of stretching of CH_2 and CH_3 of phospholipids, cholesterol and creatine [30]. The stetching of asymmetric and symmetric C-H vibrations of methyl and group of methylene are observed to be around 2930–2875 cm^{-1} [31]. The band at (2933–2923 cm^{-1}) is due to the stretching of C-H bands in malignant tissue [32]. The absorption band near (1820–1670 cm^{-1}) is due to stretch of C=O which is strong and it is the mode of stretching of lipids [33] and the band at 1636 cm^{-1} is due to C=C stretching of aromatic (vibrational mode). The prominent peak observed at 1697 cm^{-1} for stretching of C=O vibration of group of carboxyl, due to formamide [34]. The aborption peak at 1604 cm^{-1} is expected to the stretching of – C=O of ring of aromatic skeletal vibrations [35].

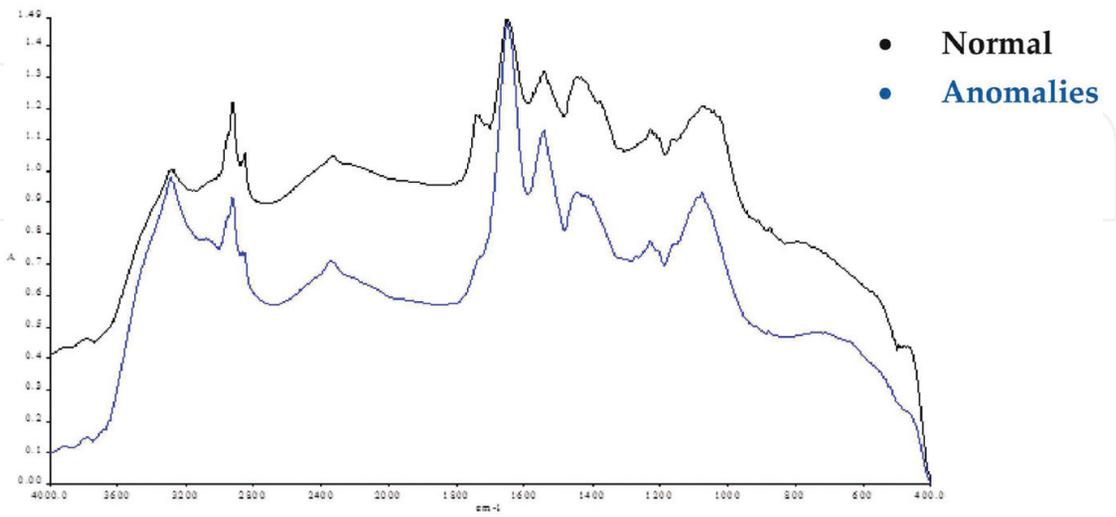

Figure 4. Comparison of saliva of normal and anomalies pregnant women.

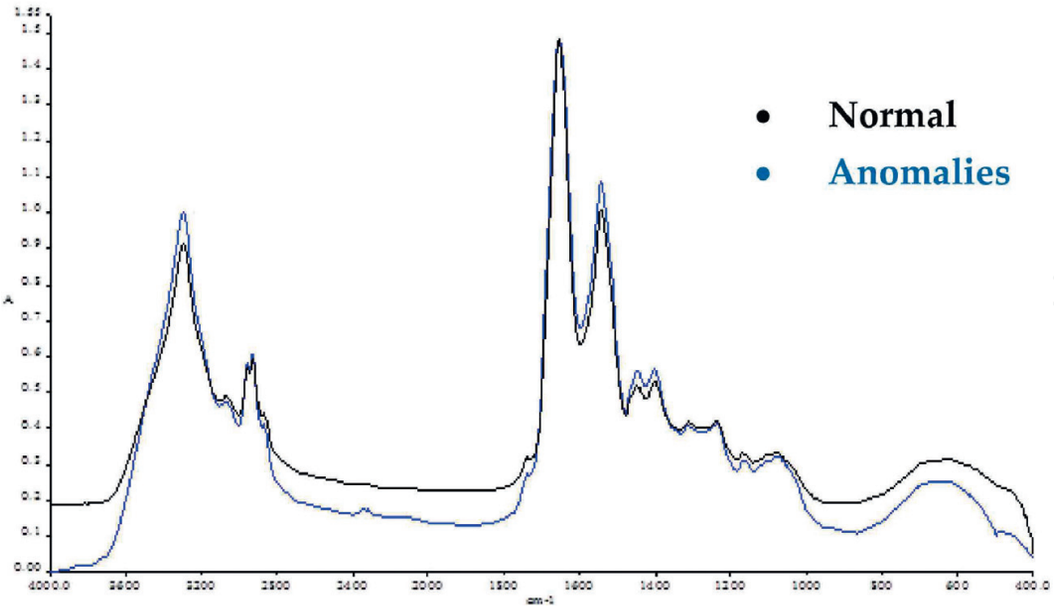

Figure 5. Comparison of serum of normal and anomalies pregnant women.

Wavenumber (cm^{-1})	Saliva		Serum	
	Normal Pregnant women	Anomalies Pregnant women	Normal Pregnant women	Anomalies Pregnant women
$R_1(I_{1338}/I_{3415})$	1.28	0.96	0.65	0.59
$R_2(I_{3546}/I_{1636})$	0.46	0.34	0.31	0.26
$R_3(I_{2854}/I_{3415})$	1.23	0.96	0.69	0.58
$R_4(I_{1743}/I_{3546})$	1.77	1.48	0.81	0.76
$R_5(I_{1482}/I_{1511})$	0.95	0.85	0.62	0.57
R_6 (I_{1482}/I_{1696})	1.01	0.91	0.73	0.68
$R_7(I_{1135}/I_{1607})$	0.89	0.81	0.47	0.40
$R_8(I_{2927}/I_{1607})$	0.97	0.92	0.90	0.85
$R_9(I_{2927}/I_{1604})$	0.98	0.94	0.92	0.87
$R_{10}(I_{1492}/I_{1511})$	0.96	0.89	0.70	0.66

Table 1. Comparative analysis of saliva and serum of normal and anomalies pregnant women.

The prominent absorption band around (1300–1000 cm^{-1}) i.e., (1150–1070 cm^{-1}) is because of stretch of C-O [29]. The peak of absorption in the region of 1480–600 cm^{-1} is relating to the band of amide II in tissue proteins. Amide II essentially comes from the stretching of C-N and bending of C-N-H vibrations feebly or weakly coupled to the stretching of bond of C=O mode. Aromatic amines shows strong stretching of C-N absorption in the region 1342–1266 cm^{-1}. The absorption shows up at higher frequencies than the corresponding absorption of aliphatic amines due to force constant of bond of C-N which is increased by resonance with a ring. Results demonstrated that there is a significant contrasts between the level of serum and saliva samples of normal and anomalies pregnant women of mid IR spectroscopy to all studied groups.

The comparison graph between normal and anomalies samples of saliva of pregnant women is shown in **Figure 4**. Samples of Serum of normal and anomalies pregnant women which are shown in **Figure 5**. A striking spectral difference observed between the samples of serum and saliva. A precise and systematic approach has been made by utilizing FTIR spectroscopic technique to study the spectral difference between healthy normal and anomalies pregnant women by utilizing saliva and serum samples and furthermore to find the efficacy of anomalies (open neural tube) defects in the embryo or fetus and absence of folate present in the pregnant women. The internal standard among the absorption peaks can be computed. In order to quantify the spectral difference, and ten intensity ratio parameters R_1 (I_{1338}/I_{3415}) R_2 (I_{3546}/I_{1636}) R_3 (I_{2854}/I_{3415}) R_4 (I_{1743}/I_{3546}) R_5 (I_{1482}/I_{1511}) R_6 (I_{1482}/I_{1696}) R_7 (I_{1135}/I_{1607}) R_8 (I_{2927}/I_{1607}) R_9 (I_{2927}/I_{1604}) R_{10} (I_{1492}/I_{1511}) have been introduced and calculated as shown in **Table 1**.

8. Statistical analysis

Variance analysis was implemented to recognize the spectral variations that were statistically significant. The t-test is one of the most rapid techniques for grouping or classifying of biological data. In the current study, the t-tests were utilized to separate certain regions of the FT-IR spectra analyzed or examined normal saliva and serum samples of healthy normal pregnant women and saliva, serum samples of anomalies pregnant women. For whole range of spectrum from 400 to 4000 cm^{-1}, analysis of statistical was performed by t-test and it shows the full effective classification to recognize or distinguish healthy normal and anomalies saliva, serum samples of pregnant women. In the analysis of t-test, considering the analysis of mean difference variance of the analysis, the t-test was carried out suggesting that the analysis of saliva investigation is better contrasted or compared with the analysis of serum. The absorbance values observed which gives a macroscopic value difference as contrasted to the minute variance observed in the analysis of serum utilized by FTIR. The intensity ratio parameters of saliva and serum samples of healthy and anomalies pregnant women are as follows: R_1 (I_{1338}/I_{3415}), R_2 (I_{3546}/I_{1636}), R_3 (I_{2854}/I_{3415}), R_4 (I_{1743}/I_{3546}), R_5 (I_{1482}/I_{1511}), R_6 (I_{1482}/I_{1696}), R_7 (I_{1135}/I_{1607}), R_8 (I_{2927}/I_{1607}), R_9 (I_{2927}/I_{1604}), R_{10} (I_{1492}/I_{1511}).

Utilizing FT-IR, saliva and serum samples were analyzed and the outcome of the results were statistically analyzed and compared using the t-tests. The standard deviation and the mean

Variance was identified and showed that the intensity ratio of saliva which predicts a good result analysis as compared with the analysis of Serum samples by utilizing FT-IR Spectrum (**Table 2**).

The t-test result outcomes were analyzed and prove that from the two samples of variances normal and anomalies pregnant women, the Saliva test gives an easier variance analysis for identification of anomalies as reviewed or explored through the measurable or statistical t-test (**Tables 3**–**8**).

The mean value is observed through the distribution of 't' at confidence interval of 95% which shows that the mean of saliva is more suitable for analysis of anomalies pregnant women as compared to analysis of serum through the correlation or comparison of intensity ratio parameters. The confidence intervals calculation and tests significance, the values of df which is associated with the unequal condition of variance are adjusted and rounded off to the closest integer.

This test is utilized for correlating the means for two samples, regardless of whether they have unequal replicate numbers. In basic terms, contrast between the actual difference between two means in connection to the variation in the data which is expressed as the standard deviation of the distinction between the methods and means utilizing the t-test.

Statistical tests take into account making statements with a higher degree level of exactness, however can't really proving or disproving anything. Significant outcome of the result at the 95% probability level make perfect data, which is adequate to help a conclusion with 95% confidence (however there is a 1 of every 20 chance of being wrong). In Biological work, to maintain and acknowledges and accepts this level of significance as being reasonable.

Saliva Analysis		Serum Analysis	
Mean difference of absorbance values	0.568	Mean Difference of absorbance values	0.054
Standard Deviation	0.19458	Standard Deviation	0.02319
Standard Error	0.06153	Standard Error	0.00733

Table 2. Statistical analysis-I.

	Saliva analysis	Serum analysis	Total
n	10	10	20
$\sum X$	5.6800000	0.5399999	6.2200000
$\sum X^2$	3.5669999	0.0340000	3.6009999
SS	0.3408	0.0048	1.6666
Mean	0.568	0.054	0.311

Table 3. Statistical analysis-II.

t-test assuming equal Sample Variances					
$Mean_a$-$Mean_b$	t	Df		one-tailed	<.0001
0.514	+8.29	18	p	two-tailed	<.0001

Table 4. Statistical analysis-III.

F-Test for the significance of the difference between the variance of the two samples			
df_1	df_2	F	P
9	9	70.4	<.0001

Table 5. Statistical analysis-IV.

t-test Assuming Unequal Sample Variances					
$Mean_a$-$Mean_b$	t	Df		one-tailed	<.0001
0.514	+8.29	9.26	p	two-tailed	<.0001

Table 6. Statistical analysis-V.

Confidence Intervals			
	Observed	0.95	0.99
$Mean_a$	0.568	0.1391	0.2
$Mean_b$	0.054	0.0166	0.0238
$Mean_a$-$Mean_b$ [Assuming equal sample variances]	0.514	0.1301	0.1785
$Mean_a$-$Mean_b$ [Assuming unequal sample variances]	0.514	0.14	0.2014
Independent Samples			

Table 7. Statistical analysis-VI.

Options consider:	
One sample analysis Equal variance Confidence intervals Mean 1: 0.568 Mean 2: 0.054 Std Dev.1: 0.19458 Std Dev.2: 0.00733	Two numbers of cases given. Mean1 eq: 0.568; mean2 eq: 0.054 Population standard deviation estimated using sample T-distribution used Difference between means: 0.514se=0.0024 95% CI of difference: 0.5092 <0.514< 0.5188 (Wald) t= 210.4; df= 9; p= 1

Table 8. Statistical analysis-VII.

9. Analysis with histogram

The bar diagram which is shown in **Figures 6** and **7** between the intensity ratio parameters and the values of absorbance were obtained from the spectra of FT-IR. The histogram clearly

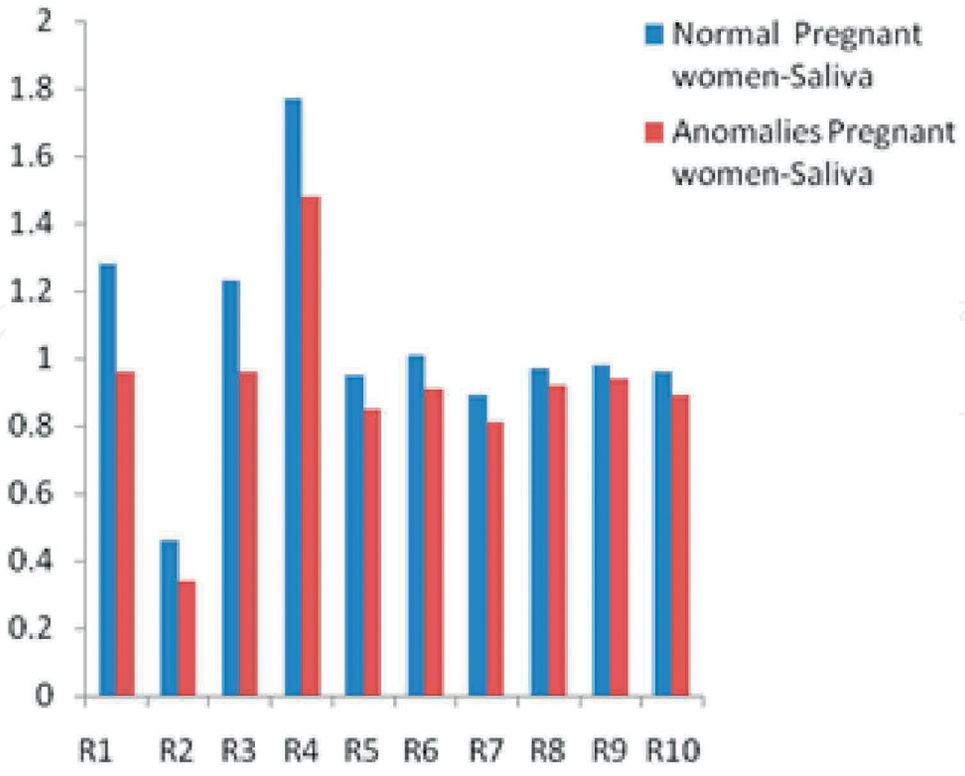

Figure 6. Comparison of intensity ratio parameters of saliva normal pregnant women and anomalies pregnant women.

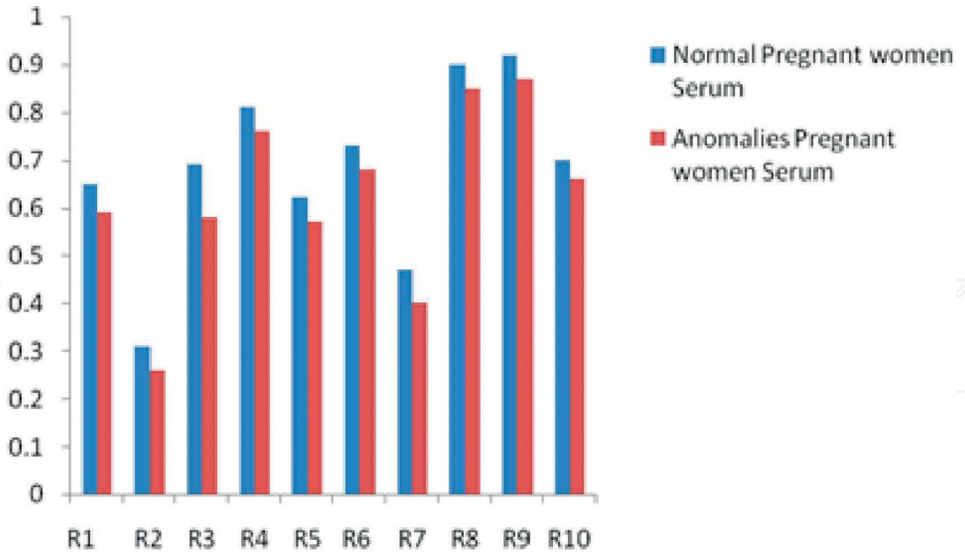

Figure 7. Comparison of intensity ratio parameters of serum normal pregnant women and anomalies pregnant women.

picture out and shows a striking contrast between the normal and anomalies pregnant women for both saliva and serum sample.

10. Conclusion

With FT-IR spectroscopy, biochemical changes and the spectral difference of both serum and saliva of normal healthy pregnant women and anomalies (open neural defect) in pregnant women are compared and detected. It is concluded that the diagnostics of saliva have a high potential to revolutionize the generation next, and offer a simple, inexpensive, and noninvasive approach for diseased detection.

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Abbreviations

FT	Fourier transform
FTIR	Fourier transform infrared spectroscopy

IR	infrared spectroscopy
LMP	last menstrual period
MSAF	maternal serum alpha fetoprotein
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
ZPD	zero path difference

A. Appendix and Nomenclature

This appendix presents the definition of terms/notation used throughout the chapter.

Notation	Definition
FTIR	Fourier-transform infrared spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. It is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas
LMP	The last menstrual period (LMP) refers to the start date of a woman's most recent menstruation, or period as indicated by the first day of bleeding
DTGS	Deuterated triglycine sulfate detector (DTGS) is a very sensitive room-temperature detector for mid-infrared range measurements that employs temperature-sensitive ferroelectric crystals of deuterated triglycine sulfate
R1	Intensity ratio parameter 1
t-test	A t-test is an investigation of two population means which implies using statistical examination, a t-test with two samples is commonly utilized with size of a small sample, testing the contrast or difference between the variance of two normal distribution are not known.
F-test	An F-test is any statistical test in which the statistic test has an F-distribution under the null hypothesis.
Std. Dev1	Standard deviation 1 is a quantity expressing by how much the members of a group differ from the mean value for the group.

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